complex native membrane is irreproducible for many spectroscopic methods, and the necessary use of solubilizing detergents for biochemical and biophysical characterization can alter protein stability and function. We explore the effects of such environments on Proteorhodopsin (PR), a proton-pumping membrane protein from marine bacteria. PR has significant structural and dynamical commonalities to mammalian seven-transmembrane proteins, including the G-protein Coupled Receptors (GPCRs). Furthermore, PR associates with itself in the membrane to form oligomers similarly to GPCRs, and these different forms are thought to play a significant role in function. Using fast protein liquid chromatography and optical absorption experiments we show that the hexameric state of PR in the nonionic dodecylmaltoside (DDM) detergent has a lower pKa value for the primary proton-acceptor residue than both the monomeric and dimeric protein, suggesting that the hexamer is more optimized for proton transport. We have also observed this phenomenon in the zwitterionic detergent dodecylphosphocholine (DPC). Next, we studied the environmental effects on PR's oligomeric state, using crosslinking followed by SDS-PAGE, and found hexamers in DDM, DPC, and E. coli membranes expressing PR. Additionally, we examine structure and dynamics using continuous wave Electron Paramagnetic Resonance (EPR). Site-directed spin-labeling EPR elucidates site-specific dynamics and detects short distances (<2 nm), making it useful for studying these complex environments. We thereby determine that a change in detergent type can change side-chain dynamics. Our work lends insight into the study of more complex mammalian proteins by demonstrating the effect of detergent on function, dynamics, and protein-protein complexes.

1529-Pos Board B259

Controlled Reconstitution of Integral Membrane Proteins by Detergent Extraction through Cyclodextrin Complexation

Carolyn Vargas[1], Martin Textor[1], Natalia Markova[2], **Sandro Keller**[1].
[1]Molecular Biophysics, University of Kaiserslautern, Kaiserslautern, Germany, [2]GE Healthcare, Uppsala, Sweden.

The reconstitution of purified membrane proteins from a detergent-solubilized state into lipid bilayer membranes is a prerequisite for many in vitro studies on membrane channels and transporters. Among the diverse methods used for the removal of detergent from ternary protein/lipid/detergent mixtures, detergent complexation with cyclodextrins offers a number of unique advantages. In particular, cyclodextrins sequester detergents selectively and at defined stoichiometries, which would, in principle, allow for a tight control of the reconstitution process and facilitate the rational optimization of experimental protocols. However, no systematic, quantitative studies on the complex interactions among cyclodextrins, detergents, and lipids have been reported to date. Thus, we adopted a microcalorimetric approach to thermodynamically characterize the complexation of a homologous series of alkyl maltoside detergents by various substituted β-cyclodextrins.

The binding affinity increased with alkyl chain length, as reflected by a Gibbs free energy increment of about 3 kJ/mol per methylene group. In contrast with many other complexation reactions involving cyclodextrins, detergent binding did not reveal enthalpy−entropy compensation. Instead, the increase in affinity with chain length resulted from both a more favorable entropy term and a less unfavorable enthalpy change. These correlations can be ascribed to enhanced conformational flexibility and decreased repulsion between cyclodextrin and the detergent headgroup, respectively, as the alkyl chain becomes longer. The thermodynamic data thus obtained were used to optimize the efficiency of detergent extraction from mixed micelles to form well-defined, unilamellar, and uniformly sized bilayer vesicles, and a quantitative model was established to simulate and analyze this phase transition. Finally, we combined the results from experiment and theory to develop new protocols for the online monitoring of the reconstitution process to aid the functional reconstitution of membrane proteins such as ion and water channels.

1530-Pos Board B260

Structure-Function Analyses Reveal *Arabidopsis Accelerated-Cell-Death11 (Acd11)* is a Ceramide-1-Phosphate Transfer Protein that Forms a Gltp-Fold

Xiuhong Zhai[1], Dhirendra K. Simanshu[2], David Munch[3], Linda M. Benson[4], H Robert Bergen[4], Helen M. Pike[1], Lucy Malinina[5], John Mundy[3], Julian G. Molotkovsky[6], Dinshaw J. Patel[2], Rhoderick E. Brown[1].
[1]Hormel Institute, University of Minnesota, Austin, MN, USA, [2]Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [3]Department of Biology, University of Copenhagen, Copenhagen, Denmark, [4]Mayo Clinic College of Medicine, Rochester, MN, USA, [5]Technology Park de Bizkaia, CIC bioGUNE, Derio, Spain, [6]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation.

The *accelerated-cell-death11 (acd11)* mutant of *Arabidopsis* provides a genetic model for studying immune response activation and localized cell suicide that halts the spread of pathogen infection in plants. Here, we elucidate ACD11 structure/function. Using a Förster resonance energy transfer approach involving anthrylvinyl and perylenoyl lipid probes, we find that ACD11 exhibits selective intermembrane transfer of ceramide-1-phosphate (C1P) and phyto-C1P, metabolites of the cell death sphingolipids, ceramide and phytoceramide. Crystal structures establish C1P binding via a surface-localized, phosphate headgroup recognition center connected to an interior hydrophobic pocket that adaptively ensheaths lipid chains via a cleft-like gating mechanism. Point mutational mapping confirmed the functional involvement of specific binding-site residues that selectively target the C1P phosphate headgroup. C1P binding selectivity also is verified by changes in fluorescence intensity associated with ACD11 intrinsic tryptophan and by ESI-mass spectrometry. Phosphatidylserine at low mole fraction in donor membranes, but not other negatively-charged phospholipids, stimulates C1P intermembrane by ACD11. A π-helix (π-bulge) present near the lipid-binding cleft distinguishes apo-ACD11 from other GLTP-folds. The global two-layer, α-helically-dominated, 'sandwich' topology displaying C1P-selective binding identifies ACD11 as the plant prototype of a new GLTP-fold subfamily. [Support: NIGMS GM45928 & NCI CA121493, Danish Strategic Research Council 09-067148, Spanish Ministerio de Ciencia e Innovacion (MICINN BFU2010-17711), Russian Fdn. for Basic Research 012-04-00168; Abby Rockefeller Mauze Trust, Maloris Fnd., Hormel Fnd.]

1531-Pos Board B261

Hiv-1 Neutralizing Antibodies and Vaccine Antigen Selectively Interact with Phase-Separated Model Membranes

Gregory Hardy, Stefan Zauscher.
Duke University, Durham, NC, USA.

Evidence suggests that lipid membrane interactions with rare, broadly neutralizing antibodies (NAbs), 2F5 and 4E10, play a critical role in HIV-1 neutralization. The **objective** of this research is to understand how lipid domains contribute to 2F5/4E10 membrane interactions and antigen localization, with the ultimate vision of guiding immunogen designs. It is well established that the native viral membrane is heterogeneous, representing a mosaic of lipid rafts and protein clustering. However, the size, physical properties, and dynamics of these regions are poorly characterized and their potential roles in HIV-1 neutralization are also unknown. **Methods:** To understand how lipid domains contribute to 2F5/4E10 membrane interactions, we have engineered biomimetic supported lipid bilayers (SLBs) and are able to use atomic force microscopy to visualize membrane domains, antigen clustering, and antibody-membrane interactions.

Our **results** showed that localized binding of HIV-1 antigens ($MPER_{656}$) and NAbs were observed to interact preferentially with the most fluid membrane domain. This supports the theory that NAbs may interact with regions of low lateral lipid forces that allow antibody insertion into the bilayer. Presence of antigen within gel-liquid disordered bilayers prevented coalescence of gel domains while antigen within the model HIV-1 bilayer prevented observable domain formation altogether.

Our research is **significant** in that it will help understand how vaccine antigen, MPER, is distributed in bilayers that model vaccine liposomes and the HIV envelope. It will also help understand the lipid environment most favorable for membrane-antibody interactions in the absence of antigen; which occurs during the first step of 2F5/4E10's proposed neutralizing mechanism.

1532-Pos Board B262

Spectroscopic Studies of Membrane Structure and Interactions of α-Synuclein 71-82

Laurie Bédard, Émilie Morin-Michaud, Thierry Lefrèvre, Normand Voyer, Michèle Auger.
Université Laval, Québec, QC, Canada.

Parkinson's disease is a neurodegenerative disease affecting approximately 100,000 people in Canada, including 25,000 in Quebec. Symptoms such as tremor at rest, rigidity, balance problems, akinesia and bradykinesia, come from the drastic reduction of dopamine. Currently, prescription drugs only reduce these symptoms, but do not stop or slow down the progression of the disease. Several studies have demonstrated the presence of aggregates in the nervous tissues, specifically in the presynaptic terminals. These aggregates cause tissue degeneration. They are made of an amyloid protein called α-synuclein which, depending on the conditions, adopts a structure rich in β sheets and forms protofibrils and fibrils. It comprises 140 amino acids including a hydrophobic core. This section plays a crucial role in the aggregation of the protein. It has been shown that amino acids 71-82 are essential for fibril

formation (71VTGVTAVAQKTV82). In the present study, we have investigated the membrane interactions of this 12-mer peptide using 31P solid-state NMR spectroscopy as well as infrared spectroscopy while the peptide structure was studied by infrared spectroscopy. The phospholipids used have different acyl chain lengths and different polar headgroups. The results show that the peptide tends to aggregate and specifically interacts with the negatively charged membranes.

1533-Pos Board B263
Cholesterol Binding Drives Partitioning of the Amyloid Precursor C99 Protein Into Liquid Ordered Membrane Domains
Paul J. Barrett[1], Charles A. Day[2], Yuanli Song[1], Jonathan P. Schlebach[1], **Anne K. Kenworthy**[2], Charles R. Sanders[1].
[1]Biochemistry, Vanderbilt University, Nashville, TN, USA, [2]Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.
The association of the 99 residue transmembrane C-terminal domain (C99) of the amyloid precursor protein with cholesterol-rich membrane domains (''lipid rafts'') is thought to promote cleavage of C99 to release the Alzheimer's disease-promoting amyloid-β (Aβ) polypeptide. Here, we test the hypothesis that C99 has an intrinsic affinity for raft-like liquid ordered (Lo) domains and that its domain preference is regulated by cholesterol binding. Fluorescence microscopy shows that C99 undergoes efficient partitioning into Lo domains relative to the co-existing liquid disordered (Ld) phase in giant unilamellar vesicles, establishing C99 as the transmembrane protein with the highest affinity for Lo domains known to date. Lo partitioning is prevented upon mutating residues that are critical for cholesterol binding to C99, indicating that binding of this sterol is a critical determinant of the phase preference of the protein. Addition of coprostanol, a raft-breaking sterol that can compete with cholesterol for binding to C99, shifts partitioning away from the Lo phase. These findings identify cholesterol binding as a novel mechanism that controls the raft targeting of C99 and suggest that pharmacological inhibition of this process could potentially be exploited as a therapeutic approach to avoid or treat Alzheimer's disease.

1534-Pos Board B264
How Do Lipids Localize in Lewy Bodies?
Himanshu Chaudhary[1], Vinod Subramaniam[2,1], Mireille Claessens[1].
[1]Science and Technology, University of Twente, Enschede, Netherlands, [2]Nanoscale Biophysics, FOM Institute AMOLF, Amsterdam, Netherlands.
Lewy bodies are the pathological hallmark of Parkinson's disease (PD). While fibrillar α-synuclein (αS) is the main protein component of Lewy bodies, these structures also contain lipids. To elucidate the presence of lipids in Lewy bodies, we investigated the interaction of lipids with monomeric and fibrillar αS. In vitro, lipid membranes accelerated αS fibril formation under physiological conditions. Moreover lipids and small vesicles co-localized with suprafibrillar structures and individual αS fibrils suggesting that aggregation initiates at the membrane. The presence of lipids in Lewy bodies may therefore be an indication that cell membranes are the major target in aggregation induced neuronal cell death.

1535-Pos Board B265
Interplay Between Amyloid Beta-Peptide and Cholesterol in Bilayer
Durgesh Rai, Divina Anunciado, Hugh O'Neill, Volker Urban, William Heller, Shuo Qian.
Oak Ridge National Laboratory, Oak Ridge, TN, USA.
The interaction between lipid bilayers and amyloid beta-peptide (Aβ) is of great interest in understanding Alzheimer's disease (AD). Accumulating evidence points to a positive association of cholesterol in the membrane and AD, but a molecular-level interaction between cholesterol and Aβ has not been established. As an essential part of the membrane, cholesterol enhances the fluidity of the lipid bilayer, which may reduce membrane permeation caused by Aβ, potentially alleviating its ability to rupture the membrane. On the other hand, cholesterol increases the binding affinity of Aβ to model lipid membranes, as Aβ shows little affinity to cholesterol-free membranes. We studied the Aβ conformations change in a model lipid membrane of DMPC/DMPG/cholesterol by circular dichroism, isothermal titration calorimetry and fluorescence. The structural changes in lipid bilayers caused by Aβ was studied by grazing angle neutron diffraction (GAND) on multilamellar lipid membrane samples in conjunction with solution Small-Angle Neutron Scattering (SANS) on lipid vesicles. The experiments combine to provide new molecular level details about how cholesterol and Aβ interact in the lipid membrane.

1536-Pos Board B266
Mimicking Lysosomal Degradation of α-Synuclein
Jennifer C. Lee, Ryan P. McGlinchey, Mercedes Vargas.
Laboratory of Molecular Biophysics, National Heart, Lung and Blood Institute, Bethesda, MD, USA.
Pathological accumulation of α-synuclein (α-syn) is a feature of Parkinson's disease. This accumulation may be counteracted by mechanisms of protein degradation that involve the proteasome and lysosome. Specifically, the lysosomal protease, Cathepsin D (CatD), has been suggested to be the main enzyme involved in the degradation of α-syn in vivo. In vitro, only C-terminal truncated species are generated, arguing that other mechanisms are needed to fully explain α-syn degradation. Here, we show that N-terminally acetylated α-syn also generates C-terminal as well as N-terminal truncated variants in the presence of CatD. These species are shown to be more aggregation prone. Since α-syn associates with membranes, we have investigated the effects of various glycosphingolipids such as glucosylceramide (GlcCer), on CatD degradation of α-syn. It is known that GlcCer buildup is a hallmark of the lysosomal storage disorder Gaucher disease and that these patients have an increased risk of developing PD. Our data clearly shows that in the presence of GlcCer and CatD, α-syn is completely proteolyzed. These data offer new mechanistic insight into α-syn degradation in the lysosome.

1537-Pos Board B267
Small Angle Scattering of Fibrinogen Polymerization Kinetics and of Alpha1 Antitrypsin Interactions with Lipid Membranes
Luis A. Palacio[1], Christopher B. Stanley[2], Soenke Seifert[3], Horia I. Petrache[1].
[1]Physics, Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [3]Chemical and Materials Science X-ray Science Division, Argonne National Laboratory, Argonne, IL, USA.
Fibrinogen and Alpha 1-Antitrypsin (A1AT) are plasma glycoproteins with different, but specific functions. A1AT has been shown to have protective roles of lung cells against emphysema, a disease characterized by lung tissue destruction [1], while fibrinogen is a major factor in the blood clotting process. Most known glycoproteins have been shown to play a role in cellular interactions but the exact role of the glycan chains is still under investigation. Previous electrophysiological measurements show that A1AT has a strong affinity to lipid bilayers perturbing the function of ion channels present in the membrane. This study was designed to investigate how protein-membrane and protein-protein interactions affect the native conformation of the protein and membrane in question. Two different glycoproteins were used for comparison purposes. For A1AT, we performed contrast-matching small-angle neutron scattering (SANS) and small angle x-ray scattering (SAXS) experiments to study the structural changes of the glycosylated form of A1AT in the presence of three different lipid membranes: POPC, POPS and DLPC. For fibrinogen, we performed dynamic light scattering (DLS) measurements to find a suitable protein concentration that would yield the reaction rate needed for a time resolved SANS study of the structural evolution of fibrinogen polymerization in solution. Guinier fits were used as a first approximation to obtain the radius of gyration (Rg) of A1AT, fibrinogen and fibrin. Pair distribution functions were used to monitor the shifts in structural parameters and Bragg peaks were used to study the structural changes of lipid vesicles. We observed that the A1AT interacts with unilamellar vesicles and that fibrin structure is affected by its polymerization rate. [1] Petrusca, et al., JBC 2010.

1538-Pos Board B268
Novel Properties of the Smurf1 C2 Domain in Cellular Lipid Binding
Jordan L. Scott[1], Robert V. Stahelin[2,1].
[1]Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA, [2]Biochemistry and Molecular Biology, Indiana University School of Medicine, South Bend, IN, USA.
Ubiquitin ligases are essential regulators of cellular homeostasis and have been shown to contribute to cancer metastasis and new virus formation. The Nedd4 family of E3 ubiquitin ligases target a number of cellular substrates including regulators involved in TGFβ growth signaling and cellular motility. This 9-member family of ubiquitin ligases has WW domains for target recognition and C-terminal HECT catalytic domains for the covalent linkage of ubiquitin to substrates. In addition, these proteins have N-terminal C2 domains that bind lipids and enable them to localize to membranes.
We have discovered new properties of the C2 domain of the Nedd4 family member Smurf1. The Smurf1 C2 domain acts as both a specific phospholipid-binding protein and as an anionic charge sensor in vitro and in